

Preoperative Serum Vascular Endothelial Growth Factor Levels: Significance in Ovarian Cancer¹

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ABSTRACT

Purpose: To assess the clinical relevance of serum vascular endothelial growth factor (VEGF) levels in distinguishing patients with ovarian cancer from those with benign adnexal masses.

Experimental Design: Preoperative serum VEGF levels were assessed in 101 women with invasive epithelial ovarian cancer, 16 with low malignant potential (LMP) ovarian tumors, and 34 women with benign ovarian tumors. VEGF levels were determined using an ELISA (R&D Systems, Minneapolis, MN).

Results: Ovarian cancer patients had a mean preoperative VEGF level of 549 pg/ml (median 379 pg/ml), which was significantly higher than those with benign adnexal masses (mean 228 pg/ml, median 155 pg/ml; $P < 0.001$) and LMP tumors (mean 200 pg/ml, median 129 pg/ml; $P < 0.001$). There were no significant differences in VEGF levels between individuals with benign masses and LMP tumors. The ability of VEGF to differentiate malignancy from benign masses at a cutoff VEGF level of 246 pg/ml gave a sensitivity of 74%, a specificity of 71%, a positive predictive value of 88%, and a negative predictive value of 48%. VEGF levels were also significantly higher in patients with stage I ovarian cancer compared with those with benign disease or

LMP tumors. Among patients with ovarian cancer, there were no significant differences in VEGF levels based on age, stage, grade, or level of cytoreduction. The presence of ascites was associated with a significantly higher VEGF level (mean 667 pg/ml, median 445 pg/ml versus mean 317 pg/ml, median 293 pg/ml; $P < 0.001$). Various preoperative VEGF levels were assessed as a predictor of survival, and a VEGF level >380 pg/ml was associated with a hazard ratio of 2.13 ($P = 0.009$) by univariate analysis. In multivariate analysis of age, stage, cytoreduction, preoperative CA-125, grade, ascites, and VEGF levels above 380 pg/ml, only VEGF levels >380 pg/ml (hazard ratio 2.33; $P = 0.02$) and advanced stage (hazard ratio 9.03; $P = 0.004$) were significant.

Conclusions: Preoperative VEGF levels may be useful in differentiating benign adnexal masses from malignancy. Preoperative VEGF levels >380 pg/ml are an independent risk factor for death because of disease.

INTRODUCTION

Ovarian cancer remains the most common cause of death among gynecologic malignancies. The reason for the high mortality is primarily because of the widely metastatic disease in most patients at the time of initial presentation. Because of poor survival associated with most ovarian cancer patients, identification of potential factors that contribute to tumor growth and metastasis is critical. It is now well recognized that tumors require a blood supply for survival, growth, and metastasis. In 1971, Folkman (1) proposed that neovascularization of a tumor was required for growth beyond 1–2 mm. Since then, many growth factors that stimulate angiogenesis have been discovered. VEGF³ is a direct angiogenic molecule and plays an essential role during embryogenesis, physiological angiogenesis, and neovascularization of malignancy (2). VEGF stimulates endothelial cell migration, proliferation, and proteolytic activity (3).

VEGF has been implicated in the pathogenesis of ovarian cancer (4). Paley *et al.* (5) have reported that overexpression of VEGF by *in situ* hybridization in early stage ovarian cancer is associated with a shortened disease-free survival. Yamamoto *et al.* (6) found similar results in patients with all stages of ovarian cancer using immunohistochemistry for identification of VEGF. Elevated serum VEGF levels have been described in patients with metastatic ovarian cancer (7), but there are little data regarding the utility of serum VEGF levels in predicting clinical and surgical outcome. Thus, we undertook this study to evaluate the utility of preoperative serum VEGF levels in predicting clinical and surgical outcome of patients with ovarian cancer. We also evaluated the utility of preoperative VEGF levels in distinguishing malignant from benign disease.

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³ The abbreviations used are: VEGF, vascular endothelial growth factor; LMP, low malignant potential.

Table 1 Comparison of VEGF levels^a

Group	n	Mean	Median	Range
Ovarian cancer	101	549	379	31–1997
LMP	16	200	129	26–539
Benign	34	228	155	11–1565

^a VEGF levels in pg/ml.

MATERIALS AND METHODS

The medical records of 151 patients evaluated and treated by the gynecologic oncology service at The University of Iowa Hospitals and Clinics between 1995 and 2000 with preoperative serum samples were reviewed. All of the samples were collected in compliance with the requirements of the Institutional Review Board for the protection of human subjects. Blood was drawn at the preoperative visit, and serum was stored at -80°C until examination. Serum VEGF levels were determined using a commercially available assay through R&D Systems (Quantikine Human Vascular Endothelial Growth Factor Immunoassay; R&D Systems) according to the manufacturer’s protocol. Patients with epithelial ovarian cancer, fallopian tube cancer, primary peritoneal cancer, LMP tumors, and benign adnexal masses were included in this study.

Patient charts were reviewed to obtain data regarding age, diagnosis, histology, grade, International Federation of Gynecologists and Obstetricians stage, presence or absence of ascites, residual disease, operative findings, timing of recurrence, and demise. Optimal cytoreduction was defined as <1-cm residual disease after cytoreductive surgery. All of the patients were surgically staged according to the International Federation of Gynecologists and Obstetricians staging system. The pathology for all of the patients with cancer was reviewed by a gynecologic pathologist. Adjuvant therapy was determined by the operating gynecologic oncologist (3 patients with stage IA ovarian cancer did not receive adjuvant chemotherapy; all of the other patients with invasive ovarian cancer were treated with adjuvant paclitaxel and platinum chemotherapy). Long-term follow-up was available on all of the patients. The status of each patient was recorded as alive without disease, alive with disease, dead of disease, or dead of other causes.

The Wilcoxon-Mann-Whitney test and the Kruskal-Wallis test were used to compare the VEGF distributions across subgroups of patients based on age, diagnosis, grade, stage, presence or absence of ascites, and cytoreduction. Survival analysis was performed using the Cox proportional hazards regression model; *P* < 0.05 was considered significant. Patients who were alive at last follow-up were censored at the date of last follow-up. Exploratory statistical methods were used to determine the optimal cutoff value for VEGF in univariate analyses. The exploratory analysis involved identifying the deciles of the VEGF distribution and then examining the hazard ratios estimated for each of the nine possible dichotomizations. The dichotomization that yielded the largest hazard ratio was selected as the optimal cutoff value for VEGF. This technique will generally give the lowest *P*; hence, statistical significance for VEGF in the univariate analyses should not be interpreted in the usual way. Ninety-five percent confidence intervals for the sensitivity, specificity, positive predictive value, and negative

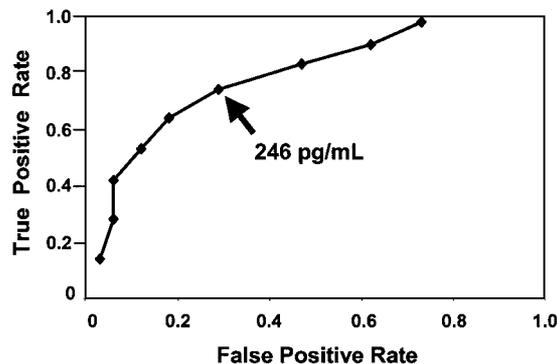


Fig. 1 Receiver operator characteristic curve for the ability of preoperative serum VEGF levels in predicting malignancy.

predictive value were based on the normal approximation to the binomial distribution.

RESULTS

There were a total of 101 patients with invasive cancer: 81 had epithelial ovarian cancer, 13 had primary peritoneal cancer, and 7 had fallopian tube cancer. These patients were combined for subsequent analyses. Sixteen patients had LMP ovarian tumors, and 34 had benign adnexal disease. Descriptive statistics for VEGF levels by group are shown in Table 1. There was an overall significant difference in the VEGF levels between the three groups (*P* < 0.001). There was also a significant difference between cancer patients and those with benign disease (*P* < 0.001), and between cancer patients and those with LMP tumors (*P* < 0.001). However, there was no difference in VEGF levels between patients with LMP tumors and those with benign disease (*P* = 0.97).

To evaluate the utility of preoperative VEGF levels in predicting malignancy, sensitivity and specificity calculations were performed for various cutoff values of VEGF in predicting malignancy using all of the patients with invasive cancer *versus* all of the patients with benign disease. The receiver operating characteristic curve is shown in Fig. 1. At a cutoff VEGF level of 246 pg/ml, the sensitivity was 74%, the specificity was 71%, the positive predictive value was 88%, and the negative predictive value was 48% for predicting malignancy. Similar results were found when patients with invasive cancer were compared with those with LMP tumors and benign adnexal disease. At a VEGF cutoff level of 246 pg/ml, the sensitivity was 74%, the specificity was 68%, the positive predictive value was 82%, and the negative predictive value was 57% for predicting malignancy. The same calculations were performed for CA-125 levels. For all of the patients with invasive cancer *versus* all of the patients with benign disease, the sensitivity was 90%, the specificity was 71%, the positive predictive value was 93%, and the negative predictive value was 68%, using a CA-125 cutoff at 35 units/liter. For all of the patients with invasive cancer *versus* all of the patients with LMP tumors and benign adnexal disease, using a cutoff CA-125 of 35 units/ml, the sensitivity was 93%, the specificity was 60%, the positive predictive value was 83%, and the negative predictive value was 72%.

Table 2 Characteristics of cancer patients and associated VEGF levels^a

Variable	n	Mean	Median	Range	P
Age					
≤64	48	542	378	49–1884	0.84
>64	53	556	382	31–1997	
Grade					
Low (1 and 2)	39	588	382	31–1997	0.92
High (3)	61	528	379	71–1689	
Stage					
Low (I and II)	20	576	403	106–1884	0.88
High (III and IV)	81	543	379	31–1997	
Cytoreduction					
Optimal	68	511	369	49–1997	0.15
Suboptimal	33	629	483	31–1689	
Ascites					
Absent	34	317	293	31–746	<0.001
Present	67	667	445	71–1997	

^a VEGF levels in pg/ml.

Next, we assessed the combined predictive ability of VEGF and CA-125. Using a VEGF cutoff of 246 pg/ml and a CA-125 cutoff of 35 units/ml, when both markers are elevated, the positive predictive value was 90%, the negative predictive value was 82%, the sensitivity was 70%, and the specificity was 39%. When either marker is elevated, the sensitivity improved to 96%, the specificity was 39%, the positive predictive value was 79%, and the negative predictive value was 82%.

To determine whether VEGF levels could be useful for differentiating benign adnexal masses from early stage ovarian cancer, the distribution of VEGF levels among patients with stage I ovarian cancer was compared with those with benign disease. There were 14 patients with stage I ovarian cancer with a mean VEGF level of 608 pg/ml (median = 403 pg/ml; range 106–1884 pg/ml), and the 34 patients with benign disease had a mean VEGF level of 228 pg/ml (median = 155 pg/ml; range 11–1565 pg/ml). The difference between these VEGF distributions is significant ($P = 0.002$) based on the Wilcoxon rank sum test. This association was also significant when patients with stage I invasive ovarian cancer were compared with patients with LMP tumors or benign disease ($P = 0.001$). Among the patients with benign disease who had relatively high preoperative VEGF levels, 1 had Meigs' Syndrome (871 pg/ml) and another had an ovarian torsion (1565 pg/ml).

Among patients with invasive ovarian cancer, the mean age was 64 years (range, 20–78 years). Thirty-nine patients (39%) had low-grade (1 or 2) disease, and 61 patients (61%) had high-grade (3) disease. Twenty patients (20%) had low stage (I or II) disease, and 81 patients (80%) had advanced stage (III or IV) disease. Sixty-eight patients (67%) underwent optimal cytoreduction, and 33 patients (33%) underwent suboptimal cytoreductive surgery. Thirty-four patients (34%) had no evidence of ascites, and 67 (66%) had ascites present. Table 2 shows descriptive statistics for preoperative VEGF values by patient characteristics among those with invasive ovarian cancer. VEGF levels were significantly higher in patients with ascites (median 445 pg/ml *versus* 293 pg/ml; $P < 0.001$). There were no significant differences in VEGF levels based on age, tumor grade, stage, or ability to achieve optimal level of cytoreduction.

Table 3 Univariate analysis of survival

Variable	Hazard ratio	95% Confidence interval	P
Age	1.34	0.76–2.36	0.30
Grade 3	1.36	0.76–2.46	0.29
Stage III/IV	10.15	2.45–42.12	<0.001
Suboptimal cytoreduction	2.21	1.26–3.86	0.007
Ascites present	2.5	1.27–4.92	0.004
VEGF >380 pg/ml	2.13	1.19–3.79	0.009

Patients with VEGF levels >380 pg/ml (this cutoff value was determined by exploratory statistics as described below) had significantly higher CA-125 levels than those with VEGF levels <380 pg/ml (median 727 pg/ml *versus* 406 pg/ml; $P < 0.001$).

Hazard ratios were determined for age, tumor grade, stage, level of cytoreduction, presence or absence of ascites, and preoperative VEGF level using univariate analysis (Table 3). A VEGF cutoff level of 380 pg/ml was used for this analysis, as this was the level that maximized the estimated hazard ratio between the two groups in exploratory statistics. Advanced stage, suboptimal surgical cytoreduction, presence of ascites, and preoperative VEGF level >380 pg/ml were associated with significantly elevated hazard ratios. The survival curve, based on VEGF dichotomization (≤ 380 pg/ml *versus* >380 pg/ml) for the ovarian cancer patients is shown in Fig. 2. There were 51 patients with VEGF values ≤ 380 pg/ml and 50 with VEGF values >380 pg/ml. There was a significant difference ($P = 0.009$) in survival based on this dichotomization. The 2-year survival rate for patients with low VEGF (≤ 380 pg/ml) levels was 70.3%, and the 5-year survival rate was 48.9% (median survival 4.22 years). In contrast, for individuals with high preoperative VEGF levels, the 2-year survival rate was 57%; and the 5-year survival rate was only 23.5% (median survival 2.40 years). After adjusting for age, stage, grade, cytoreduction, and ascites in a Cox regression model, VEGF remained significantly associated with survival ($P = 0.02$). High stage was the only other factor to be significantly associated ($P < 0.001$) with survival in the multivariate model (Table 4). When preoperative CA-125 was included in the multivariate model, VEGF ($P = 0.02$) and stage ($P = 0.004$) remain the only factors significantly associated with survival.

DISCUSSION

The development and progression of cancer depends on multiple, sequential, and interrelated steps. Among these, angiogenesis is a critical step in the progression and metastasis of ovarian and other cancers. Several lines of evidence support the association between VEGF and ovarian cancer-related angiogenesis (4). Although there is abundant evidence to show that VEGF plays a central role in the development and growth of malignant tumors, there is limited information regarding the clinical utility of serum VEGF levels in ovarian carcinoma. Previous studies regarding the utility of serum VEGF levels as a predictor of survival in patients with ovarian cancer (8–10) and as a predictor of malignant *versus* benign disease (11, 12) have demonstrated mixed results. Therefore, the utility of serum VEGF as a tumor marker has not yet been established. Although

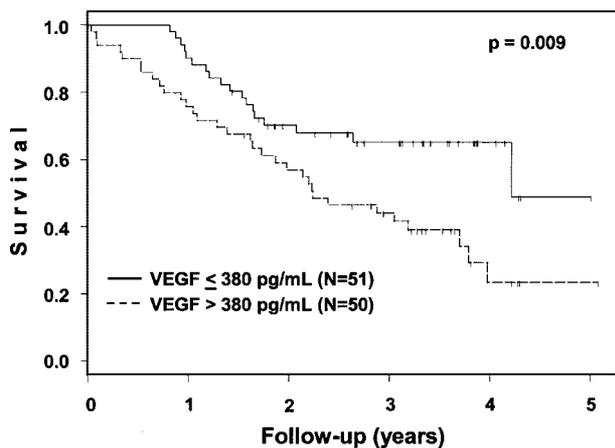


Fig. 2 Survival for patients with invasive ovarian cancer based on preoperative serum VEGF levels. Vertical tick marks on the curves indicate censored observations.

serum VEGF levels may be influenced by platelet counts, Lee *et al.* (13) have demonstrated that platelet-derived VEGF also reflects the biology of cancer cells and that serum would be more useful than plasma for measurement of circulating VEGF in cancer patients for prognosis. Thus, serum samples were used in the present study.

Kraft *et al.* (7) showed a significant difference in VEGF levels between 40 patients with ovarian cancer compared with 145 healthy individuals. They also found a significant difference in serum VEGF levels between the 5 patients with nonmetastatic disease and the 35 patients with metastatic disease (7). Another study found a significant difference in serum VEGF levels between healthy individuals and patients with ovarian cancer, but not between patients with cystadenomas and patients with ovarian cancer (11). Oehler and Caffier (12) reported a significant difference in patients with cancer compared with both healthy controls and patients with cystadenomas. Yamamoto *et al.* (6) found a significant difference in VEGF levels between patients with cancer compared with both patients with benign disease and LMP tumors. All of these studies compared patients with various stages of ovarian cancer. Gadducci *et al.* (10) compared serum VEGF levels between 53 patients with ovarian cancer and 25 patients with benign ovarian disease. They found a significant difference between all of the patients with cancer and the control group, but no difference between patients with stage I or II ovarian cancer and the controls. Reasons for various results in these studies may include differences in assay techniques, storage of specimens, and the small numbers of samples analyzed in some studies. Serum VEGF levels have also been shown to be elevated in other malignancies including brain, renal, breast, and gastrointestinal malignancies (14–17). In our study, we demonstrated a significant difference in preoperative serum VEGF levels between patients with cancer compared with both patients with benign disease or LMP tumors. Moreover, we found a significant difference between preoperative serum VEGF levels in patients with stage I ovarian cancer compared with those with benign disease. Also, >80% of the patients with mucinous cancers had elevated VEGF levels.

Table 4 Multivariate analysis of survival

Variable	Hazard ratio	95% Confidence interval	P
Age	1.16	0.64–2.10	0.63
Grade 3	0.86	0.47–1.58	0.63
Stage III/IV	9.24	2.10–40.64	<0.001
Suboptimal cytoreduction	1.29	0.70–2.37	0.42
Ascites present	1.28	0.58–2.79	0.54
VEGF >380 pg/ml	2.08	1.10–3.95	0.02

These results suggest that serum VEGF may be helpful in distinguishing invasive cancers from LMP or benign ovarian tumors, and can offer some added benefit to CA-125 levels. There were 2 patients with benign disease who had significantly elevated preoperative VEGF levels. It is tempting to speculate that the development of ascites in Meig’s syndrome may be related to elevated VEGF levels, because VEGF is known for its ability to induce vascular permeability. Hypoxia is known to result in elevated VEGF levels as well (18). It is possible that adnexal torsion may result in elevated VEGF levels because of local hypoxia. Nonetheless, both of these patients would have required surgery because of their symptoms.

VEGF levels have been shown to drop after cytoreductive surgery (6, 7) with the implication that VEGF levels may be useful in postdisease status. CA-125 has already proven effective in following the status of ovarian cancer (19). It is possible that serum VEGF levels may be a useful marker to follow for surveillance of ovarian cancer patients. VEGF levels did correlate with the presence or absence of ascites, but not age, grade, stage, or level of cytoreduction. This observation is consistent with previous reports (6, 8–10). Elevation in serum VEGF is likely to be an early event during the development of ovarian cancer, as serum VEGF levels were significantly different between patients with stage I ovarian cancer compared with those with benign or LMP ovarian tumors. It is possible that the predictive ability of preoperative VEGF levels could be additionally enhanced by other preoperative screening tools such as transvaginal ultrasound and serum CA-125 levels in differentiating pelvic masses.

Previous studies relating serum VEGF levels to survival have also produced mixed results. Some studies have found VEGF to be an independent prognostic factor using multivariate analysis (8, 9), whereas others did not find a correlation between serum VEGF and survival (10). In our study, which is the largest to date, preoperative serum VEGF levels were an independent prognostic factor in ovarian cancer. We have shown previously that preoperative CA-125 levels are an independent prognostic factor in patients with ovarian cancer (20). However, when CA-125 and VEGF are combined in multivariate analysis, only VEGF levels remained significant.

In summary, preoperative serum VEGF levels were significantly elevated in ovarian cancer patients compared with those with benign pathology or LMP tumors and may be a useful aid in predicting malignancy. This study has provided the first evidence demonstrating the clinical significance of using preoperative serum VEGF levels as an independent prognostic factor for patients with ovarian cancer.

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